REMARKS

Status of the Prosecution:

Applicants wish to thank the examiner for acknowledging the claims for foreign and domestic priority, and the receipt of the related documents. Claims 1-20 were elected in response to the restriction requirement and remain pending in the application and are presently under consideration. Claims 1-5 and 7-20 have been rejected herein. Claim 6 is objected to as dependent upon a rejected claim.

Applicants wish to advise the Examiner that corrected drawings have been sent to the Official Draftsperson under separate cover and courtesy copies are provided for the examiner herewith.

Claims 1-5 and 17 have been amended herein. Applicants submit that there is support in the specification for all claim amendments, and that these amendments present no new matter. The claims have been amended to clarify the claimed invention.

The Claims Are Patentable Over The Cited Art

Claims 1-5 and 7-20 stand rejected under 35 U.S.C. 35 U.S.C. §103(a) as allegedly unpatentable over Mitchell *et al.* (J. Biol. Chem 270:29766-72, 1995) ("Mitchell") in view of Fischhoff *et al.* (U.S. Patent 5,500,365,) ("Fischhoff"). Applicants respectfully traverse this rejection with respect to the claims as amended.

The claims are generally directed to a synthetic fatty acid desaturase gene for expression in a multicellular plant, wherein the gene comprises a fatty acid Δ-9 desaturase domain and a cyt b_5 domain, wherein the gene is customized for expression in a plant

cytoplasm. The claims are further directed to said gene wherein the gene is customized from a naturally occurring desaturase and cyt b5 domains selected from particular sources, and more particularly wherein the naturally occurring gene is: from *Saccharomyces cerevisiae* , encodes SEQ ID NO:2, or comprises SEQ ID NO:1, and to synthetic genes comprising SEQ ID NO:3, or further comprising an expression regulatory sequence from a plant encoding an ER biosynthetic pathway enzyme. The claims are also directed to methods of constructing a customized bifunctional fatty acid Δ-9 desaturase /cytb5 -encoding gene for expression in the cytosol of a multicellular plant.

The Office Action correctly states that Mitchell teaches the OLE1 gene of *Saccharomyces cerevisiae*, a Δ-9 fatty acid desaturase with a cytochrome b_5 domain. Mitchell also teaches complementation of both an OLE1-disrupted yeast mutant and an OLE1/cytochrome b5-disrupted yeast double mutant with the OLE1 gene. The Office Action alleges that Mitchell also teaches "inherent placement of a heterologous cytochrome b_5 domain thus teaching what the replacement of the native cytochrome b_5 domain should be." Applicants do not address this allegation as Applicants are unclear as to exactly what is meant by this statement. The claims do not contain a limitation directed to replacement of native cytochrome b_5. The Office Action acknowledges that Mitchell does not teach any customization. Mitchell also does not provide any teaching or suggesting relating to plants, nor the expression of OLE1 or any fatty acid desaturase in plants. Additionally, as the Office Action correctly states, Mitchell does not teach any customization of genes.

The Office Action alleges that it would have been obvious at the time of application to modify the teachings of Mitchell for expression in the cytoplasm of plants using the methods of optimizing genes for expression implants as taught and suggested by Fischhoff.

The Office Action states that Fischhoff teaches optimization of *B.t. tenebrionis* insecticidal protein sequence for expression in dicot and a monocot species. Additionally the Office Action alleges that Fischhoff suggests the optimization of any transgene for increased expression in transformed plants.

The Office Action also alleges that one of skill in the art would have been motivated by the knowledge common in the art that the fatty acid biosynthesis gene comprising a desaturase domain and a cytochrome b_5 domain optimized for expression in the cytoplasm of plants is a valuable tool for genetic engineering of plants and the success of Fischhoff in optimizing expression of *B.t. tenebrionis* insecticidal proteins would have provided a reasonable expectation of success of expressing the fatty acid desaturase genes in transformed plants and plant cells. The Office Action also alleges that the "[t]he choice of a promoter or regulatory region of a gene encoding an ER biosynthetic enzyme to express the fatty acid desaturase gene would have been an obvious choice."

As a threshold matter, Applicant would like to address the nature of the two aspects which the examiner asserts are common knowledge or obvious. Applicants respectfully submit that the assertion that "the fatty acid biosynthesis gene comprising a desaturase domain and a cytochrome b_5 domain optimized for expression in the cytoplasm of plants is a valuable tool for genetic engineering of plants" arguably derives from Applicants' disclosure and should be properly be supported by a prior art reference or an affidavit from the examiner, in accordance with MPEP 2144.03. Applicants according take this opportunity to invite the examiner to provide evidence as to this assertion. Similarly, Applicants also invite the examiner to provide evidence as to why the "[t]he choice of a promoter or regulatory region of a gene encoding an ER biosynthetic enzyme to express the fatty acid desaturase

gene would have been an obvious choice." Since these statements are critical to the rejection of one or more claims, and particularly since these statements are the sole basis provided in the Office Action of any motivation in the art, requesting proper support is both necessary and justified.

Notwithstanding the above traversal, Applicants respectfully assert that the *prima facie* case presented in the Office Action is insufficient on other grounds. In addition to at least the appearance of impermissible hindsight (see above), Applicants respectfully assert that the cited art, at best, provides an "obvious to try" rationale. The Mitchell reference provides no teachings or suggestions regarding the applicability of the OLE1 gene or any gene to plants. That Fischhoff may possibly be read to broadly suggest that his methods could be used with *any* gene does not mean that his technology is enabling for *any* gene. Fischhoff expressly states that the purpose is to provide

> "[a] method for modifying structural gene sequences to the expression of the protein product is disclosed. Also disclosed are novel structural genes which encode insecticidal proteins. . .and the coat protein of potato leaf roll virus."

Fischhoff's teachings are essentially limited to insecticidal proteins, such as from *B.t. tenebrionis*. Insecticidal proteins are well-known to be expressed in plants at various levels. Optimization of the expression makes sense in the context of such knowledge. The only other protein which Fischhoff exemplifies in a plant virus coat protein. Plant virus coat proteins are also known to be expressed in plants at varying levels. Fischhoff does not teach catalytic proteins such as those of the instant invention. Given the purpose of Fischhoff is solely to *optimize* the expression level of structural genes in plants, one of skill in the art, armed with Mitchell and Fischhoff, at best, would consider *trying to express* the fatty acid

desaturase in a plant to determine whether it can be optimally expressed, for example without toxic or deleterious effects. In other words, the problem solved by the present inventors is not simply "optimizing" the expression of a structural protein known to be expressed in a plant. One of ordinary skill in the art would have had to recognize the likelihood of success of expressing the protein at optimum levels. Unlike the structural genes Fischhoff teaches, merely increasing the expression level of gene encoding a catalytic enzyme does not necessarily work – Fischhoff cannot be read to provide any suggestion, motivation or expectation of success for "optimizing" such a protein where it is not even known if the protein can be successfully expressed in plants at such levels, and if successfully expressed, whether it could function properly catalytically. Fischhoff can provide no expectation of success in that regard.

Unlike the relatively simple requirements of optimizing expression of a structural gene such as an insecticidal protein or viral coat protein, one of skill in the art would presume, in view of the teachings of Mitchell, that the OLE1p might interfere with the proper electron transport in the pathway(s) leading to and/or from it.

Mitchell teaches that exact effects of a linked desaturase and cytochrome b_5 are unknown and that there may be deleterious effects on electron transport. For example:

> "Tethering the cytochrome b_5 to the desaturase could potentially speed up the electron transfer by presenting a correctly oriented heme group with respect to the dioxo-iron cluster, eliminating the need for diffusion and reorientation of the reduced cytochrome b_5. The linkage of the heme domain with the desaturase domain of Ole1p raises questions, however, concerning the docking sites for electron transfer among the cytochrome b_5, its electron donor, and electron acceptor." (Mitchell at 29772, lines 16-24).

And

> "We cannot rule out the possibility, however, that the residual carboxyl-terminal peptide sequences in the truncated forms of Ole1p may block the ability of the diffusibile cytochrome b_5 to participate in electron transfer that normally occurs in wild type cells." (*Id.* at 29771, column 1, line 5 through column 2, line 5).

Applicants respectfully assert that these serous questions expressly posed by Mitchell teach away from the idea of using these proteins in systems where the effects on electron transport are unknown, for example in plants. At best, they eliminate any *expectation* of success. This is clearly an invitation to experiment. Given that there is no teaching, suggestion or motivation in Mitchell which might relate to plants or provide an expectation of success in plants, Applicants respectfully submit that at best, Mitchell in view of Fischhoff provide an "obvious to try" rationale, or an invitation to experiment. That is not the standard for a *prima facie* case of obviousness. Accordingly Applicants respectfully request that the rejection be reconsidered and withdrawn.

In addition, Applicants respectfully assert that even if the *prima facie* case were sufficient, in this case, the secondary consideration of unexpected results rebuts the rejection. Assuming *arguendo* that one of skill was motivated to combine the teachings of Mitchell with the teachings of Fischhoff, he would not have expected the results attained by the Applicants, in particular the advantages of a bifunctional desaturase were not expected.

For example, the specification states "prior to the present invention, it was not appreciated that the bifunctional yeast desaturase offers a significant advantage over the single-function animal desaturase in plant cells, where the requisite cyt b_5 is available only in

small amounts, and the yeast protein can provide its own supply of cyt b_5." Specification at page 12, lines 28 through page 13, line 1.

It was also not appreciated previously, and the Applicants' discovered that even though some non-plant desaturases had been expressed in plants, that they were not expressed optimally, and optimizing expression would yield advantages. (*Id.* at page 13, lines 4-7). These advantages are furthered through the addition of sequences containing elements that improve the desaturase activity by increasing the intracellular protein targeting and or enzyme stability. (*Id.* at page 21, lines 19-22).

Accordingly, Applicants respectfully assert that even if the prima facie case were established, the secondary consideration of unexpected results is sufficient to rebut. Applicants respectfully request reconsideration and withdrawal of the rejection.

The Claims Are Adequately Described

Claims 1-5 and 7-10 stand rejected under 35 U.S.C. 112, first paragraph, as allegedly containing subject matter which was not described in the specification is such a way as to reasonably convey to one of skill in the art that, at the time of filing, the inventors had possession of the of the claimed invention. Applicants respectfully traverse the rejection with respect to the claims as amended.

The Office Action acknowledges that the Applicants have described a fatty acid desaturase gene from *Saccharomyces cerevisiae* of SEQ ID NO:1 and a customized version of the native gene SEQ ID NO:3, both of which encode the polypeptide of SEQ ID NO:2.

The Office Action alleges that the Applicants describe no other synthetic fatty acid desaturase genes from an unspecified source comprising a desaturase domain and a

cytochrome b5 domain customized for expression in a plant cytoplasm. The Office Action further alleges that in view of the breadth of the claims and lack of guidance, the specification does not provide an adequate written description of the claimed invention.

The Office Action also cites *Regents of the Univ. of Cal. v. Eli Lilly & Co.* (43 USPQ2d 1398 (Fed. Cir. 1997) for the proposition that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism, despite the disclosure of a cDNA encoding that protein from another organism. The Office Action further cites the same court as stating that a description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus.

In the instant case, Applicants amended claims are directed to a synthetic fatty acid desaturase gene comprising a desaturase domain of a fatty acid Δ-9 desaturase and a cyt b_5 domain, wherein the gene is customized for expression in a plant cytoplasm.

The adequacy of a written description is a question of fact which must be determined on a case-by case basis. MPEP 2163. A written description is given a strong presumption of adequacy and rejection of original claims for lack of written description should be rare. Id. An examiner must overcome the presumption of adequacy by putting forth, on a reasonable basis, sufficient evidence or reasoning. *In re Wertheim*, 541 F.2d 257, 263 (CCPA 1976). Arguing lack of literal support is not enough since the invention need not be described in *ipsis verbis* to satisfy the written description requirement. Id. at 265.

As the Federal Circuit has stated: ". . .the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." *Vas-Cath Inc. et al. v. Mahurkar et al.*, 935 F.2d 1555, 1563-4 (Fed. Cir. 1991) (emphasis in original). See also *Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1566 (Fed. Cir. 1997). A preponderance of evidence is required as to why a skilled artisan would not recognize a description of the claimed invention, as that is the perspective from which satisfaction of the requirement is measured. *Amgen Inc. v. Hoechst Marion Roussel, Inc. et al.*, 314 F.3d 1313, 1330 (Fed. Cir. 2003) (citing *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997)); see also MPEP 2163. The written description inquiry, therefore, focuses on a comparison between the specification and the invention referenced by the terms of the claim. *Id.* at 1332.

Possession of the invention may be established through words, structures, figures, diagrams and formulas which fully set forth the claimed invention. *Lockwood*, 107 F.3d at 1572. "Generally there is an inverse correlation between the level of skill and knowledge in the art and the specificity of the disclosure necessary to satisfy the written description requirement." MPEP 2163.

Applicants respectfully submit that adequate description of the claims has been provided, in accordance with the guidelines. In addition to the descriptions of two members admitted by the examiner, which are the exemplary genes, the Applicants have provided also provided sufficient identifying information for multiple other genes, including GenBank Accession numbers for at least four additional sequences and two partial sequences for bifunctional desaturase/cyt b_5 enzymes which can readily be modified according the methods

and examples provided in the specification. One of skill in the art would appreciate that the inventors were in possession of these synthetic sequences as well as those exemplified. In addition, the Guidelines for Written Description for original claims state that possession may be shown by showing a reduction to practice. Here a sufficient number of representatives of the genus are required. The sequences and accession numbers, as described above are found in Figure 1, and described on page 14, lines 21 through page 15 line 6 of the specification.

The Federal Circuit has steadfastly refused to require sequences in all cases of claims to genetic material. "[M]ore recently in Enzo Biochem, we clarified that Eli Lilly did not hold that all functional descriptions of genetic material fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure." *Amgen*, 314 F.3d at 1332.

Here, the correlation between the well-characterized desaturase domains, and the well-characterized cytochrome b_5 domains (see also page 14 – 15 of the specification), in combination with the well described and exemplified steps of customization are more than sufficient to convince a skilled artisan that Applicants were in possession of the claimed invention. In particular, one of skill in the art would recognize that Applicants were in possession of the common attributes or features of the elements possessed by members of the genus based on the species disclosed and the methods for generating additional genus members from the additional sequences provided.

Additionally as the specification teaches (see page 43, lines 13-18), another embodiment was actually reduced to practice by the inventors:

> "An alternative version of *pl-ole1*, referred to herein as *pl-ole1-2*, was also constructed. This synthetic gene was modified only in specific codons identified as high frequency splicing signals. It was discovered that this construct is expressed equally as well as pl-ole1 in *Arabidopsis.*"

Given the Federal Circuit's clear guidance that functional descriptions which correlate to art-known structure, especially where combined with ample sequence information, Applicants respectfully assert that the written description is more than adequate for onew of skill in the art to conclude that the Applicants were in possession of the subject matter of claim 1. Applicants need not provide a sequence or exemplify each member of the genus where the genus is adequately described as such. Applicants have satisfied the *quid pro quo* for the patent grant. Nothing more is required under 35 U.S.C. §112, first paragraph. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

The Specification Enables One of Ordinary Skill in the Art To Make And Use the Invention

Claims 1-5 and 7-20 are rejected under 35 U.S.C. §112, first paragraph as allegedly containing subject matter which was not described in such a way as to enable one of skill in the art to make and/or use the invention. Applicants respectfully traverse this rejection with respect to the amended claims.

The Office Action alleges that the optimization of the *OLE1* sequence of SEQ ID NO:1 modified to SEQ ID NO:3 both of which encode SEQ ID NO:2 for optimal expression in *Arabidopsis*, vacuum infiltration of *Arabidopsis* with the optimized OLE1 gene, pl-ole1

from *Saccharomyces cerevisiae* (Example 2); prophetic alteration of amino acids of the coding sequence of the *OLE1* fatty acid desaturase for increasing catalytic activity of the enzyme and transposition of elements, i.e. the N and C termini, form the Arabidopsis *FAD2* gene in the coding sequence of the OLE1 fatty acid desaturase for improving modified OLE1 gene expression in a plant (Examples 3 and 4).

The Office Action further alleges that the design of synthetic genes for increased expression implants can be a costly and time consuming process of building error-free sequences. The Office Action alleges that the use of time and therefore cost consuming techniques such as PCR site-directed mutagenesis are necessary for the correction errors that will inevitably arise during the resynthesis of the gene. In particular, the examiner relies on Mazier *et al.* (*Biotech. Ann. Rev.*, 1997: 313-347 at 326 lines 12-16 and 27-34) ("Mazier") and Iannocone *et al.* Plant Molec. Biol. 34: 485-496, 1997 at 490, col. 1, lines 17-37) ("Iannocone") for supporting this position.

Applicants respectfully assert that the claims are fully enabled by the entire specification – the examples are not the limit of the teachings of the Applicants disclosure. There is no statutory, or regulatory support for attempting to limit the Applicants to the Examples to the exclusion of all else that is taught in the specification.

The question of enablement is a question of law, based on underlying factual determination. *Amgen, Inc. v. Hoechst Marion Roussel, Inc. et al.*, 314 F.3d 1313,1334 (Fed. Cir. 2003). Before any analysis of enablement can occur, it is necessary for the examiner to construe the claims. The examiner should always look for enabled, allowable subject matter and communicate to Applicants what that subject matter is at the earliest point possible in the prosecution of the application. (MPEP 2164.04)

The Federal Circuit has consistently held that "the specification must teach those of ordinary skill in the art how to make and use the full scope of the invention without undue experimentation. *In re Wright*, 999 F.2d 1557,1561 (Fed. Cir. 1993). The specification need not explicitly teach those in the art to make and use the invention; the requirement is satisfied if, given the what they already know, the specification teaches those skilled in the art enough that they can make and use the invention without "undue experimentation." *Amgen*, 314 F.3d at 1334. The fact that a quantity of experimentation, even complex experimentation, may be required is not dispositive of the analysis (MPEP 2164.04). The key word is "undue," not "experimentation". *In re Angstadt*, 537 F.2d 498,504 (CCPA 1976). The factors to be considered in determining whether experimentation is undue include the breadth of the claims; the nature of the invention; the state of the prior art; the level of one of ordinary skill; the level of predictability in the art; the amount of direction provided by the inventor; the existence of working examples; and the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). It is improper to conclude that a disclosure is not enabling based on analysis of only one of the factors while ignoring one or more of the others. MPEP 2164.01(a).

Nevertheless, not everything necessary to practice the invention need be disclosed. The Federal Circuit has stated that what is well-known is best omitted. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. See, e.g., *In re Fisher*, 427 F.2d 833, 839 (CCPA 1970). Additionally, as the Federal Circuit recently reiterated, the law is clear that the specification need teach only one mode of making and using a claimed composition. *Amgen*, 314 F.3d at 1335.

Here, the Applicants note that it is not clear whether any claim scope has been identified as enabled in the Office Action. Additionally, Applicants respectfully assert the standard for enablement also does not entail the "cost of the techniques" used, nor do the Wands factors as set forth above.

The Office Action presumably alleges that Mazier and Iannacone address the unpredictability in the art. While Mazier and Iannacone may address the predictability of making synthetic genes from *Bacillus* endotoxins, there is nothing in either reference which even remotely suggests any relation to the predictability of customizing fatty acid desaturase genes, the subject of the Applicants invention. In fact, the Office Action cited Fischhoff as a allegedly prior art reference in making the obviousness rejection. Fischhoff teaches exactly the subject matter of making synthetic genes from *Bacillus* insecticidal proteins for expression in plants. Applicants struggle to understand how Mazier and Iannacone, which deal with specific synthetic genes based from naturally-occurring B.t. genes can provide evidence of a general unpredictability in the art, when they do not even unequivocally provide evidence of widespread unpredictability among B.t. genes, in view of the issued patent to Fischhoff.

Further, Mazier states that "[t]o date, the design and synthesis of three toxin-encoding genes has been reported in the literature.") Mazier, page 326, lines 14-16. Mazier also provides Table 3 which includes an exhaustive list of successful experiments conducted with transgenic plnats expressing synthetic toxin gene. Mazier, pages 327-328. Mazier also states that more recent methods are less error-prone and require fewer steps, in part as a result of thermostable polymerases. Mazier, page 326, last (partial) paragraph through page 327, first (partial) paragraph. Finally, Mazier indicates that the expression of synthetic (codon-

optimized) genes in transgenic plants allows the production of toxin protein ranging from 0.02 to 0.5% of the leaf total soluble protein. Mazier, page 329-330 (see entire section on *Codon-optimized genes show enhanced expression in plant cells.*). Applicants respectfully submit that when viewed as a whole, Mazier does not support the unpredictability in the art, and particularly not sufficiently to outweigh the other Wands factors.

With respect to Iannacone, the findings are even more clear. Iannocone discuss problems regarding low expression levels of B.t. toxins in transgenic plants with respect to codon usage, G+C content, ATTTA sequences, polyadenylation and splicing sites recognized as destabilizing sequences or introns. (see page 485, column 2 through 486 first paragraph). Iannocone then state:

> "The strategy followed to obtain a good level of expression *in planta* has consisted in the elimination of these putative destabilizing sequences located within the coding sequence of wt Bt genes, without altering the amino acid sequence. Transgenic maize, cotton, potato, tomato, tobacco and rice have been obtained using this approach."

With their own experiments they report that fragments 1, 2, 3, 7, 8, and 9 were isolated as correct clones and no mutations were detected, difficulties arose with fragments 4, 5, and 6. Although Iannacone identified a problem with these particular sequences, they clearly attribute the cause to the crude synthetic oligonucleotide preparation that they used during the experiments. The problems were corrected by using a high fidelity *Taq* polymerase. Applicants respectfully submit that rather than support the view presented in the Office Action, Iannacone supports the proposition that the use of synthetic genes is a useful and relatively predictable method when experiments are conducted properly.

In accordance with the above discussion, Applicants respectfully assert that the Office Action has not satisfactorily established a lack of enablement of the claimed invention. Applicants respectfully submit that the Wands factors in this case support the claims as enabled. No evidence of unpredictability has been presented except as clearly refuted above. The cost- or time- consumption of any required experimentation does not appear to a direct consideration. If any experimentation is required it is clearly routine. The skill in the art is quite high, the specification as a whole and the working examples would clearly enable one of skill in the art to make and use the invention commensurate with the claims.

Applicants respectfully submit that the claims are fully enabled and request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph.

In view of the claim amendments and the foregoing evidence and discussion, Applicants believe that all claims are in condition for allowance, and the same is earnestly sought. The examiner is invited to speak with the Applicants undersigned representative at anytime at 215-557-5986.

Respectfully submitted,

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